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Oocytes, Larvae, and Cleptoparasitic Behavior of *Biastes emarginatus* (Hymenoptera: Apidae: Nomadinae: Biastini)

JEROME G. ROZEN, JR.,[1] JAKUB STRAKA,[2] AND KATERINA REZKOVA[2]

ABSTRACT

We present information on the nest-searching and parasitic behavior of the European cleptoparasitic bee *Biastes emarginatus* (Schenck), found attacking nests of *Rophites quinquespinosus* Spinola in the Czech Republic. Its mature oocyte, first instar, and last larval instar are described and illustrated by SEM micrographs, microphotographs, and diagrams. These stages are compared with those of other members of the Biastini. Because the first instar of the related *Neopasites cressoni* had not been described before, its description is appended, so that comparisons can be made with *B. emarginatus*. In most respects, the biology and immature stages of *B. emarginatus* closely resemble what is known concerning other tribal members, but we note that the mature larva, though agreeing morphologically with those of close relatives, has an anatomy that invites investigation into adaptive function.

INTRODUCTION

This study reports on the biology and immature stages of the cleptoparasitic bee *Biastes emarginatus* (Schenck) and compares the information with existing knowledge concerning other members of the Biastini, one of the numerous tribes of the Nomadinae, all of which are cleptoparasites. This small tribe of three genera totalling12 named species is restricted to the Holarctic region, with *Neopasites* and *Rhopalolemma* in North America and *Biastes* in the Palearctic (Michener, 2007). Known hosts of all biastines belong to the Rophitinae (Halictidae), as follows (host names in parentheses): *Neopasites* (*Dufourea*), *Rhopalolemma* (*Protodufourea*), *Biastes* (*Systropha*, *Rophites*, and *Dufourea*) (Michener, 2007; Westrich, 1989).

[1] Division of Invertebrate Zoology, American Museum of Natural History (rozen@amnh.org).
[2] Faculty of Science, Charles University in Prague, Department of Zoology, Viničná 7, 128 44 Praha 2, Czech Republic (straka-jakub@vol.cz).

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Beyond host associations, biological information concerning these cleptoparasitic genera is limited. Torchio et al. (1967) described host-searching behavior of adults, the eggs, egg-laying habits, and larval behavior of *Neopasites cressoni* Crawford. Rozen et al. (1997) reported on various aspects of the biology of *Rhopalolemma rotundiceps* Roig-Alsina. However, details concerning the biology of *Biastes* have been lacking.

The immature stages of these three genera have been examined on a number of occasions. Egg/mature oocytes of exemplars of all genera have been described. Most of these descriptions were accompanied by information concerning the number of ovarioles per ovary found in dissections because among the Nomadinae there is extensive variation in ovariole counts from one species to the next. Egg/oocyte descriptions are as follows: *Neopasites cressoni* (Torchio et al., 1967; Rozen et al., 1997); *Rhopalolemma rotundiceps* (Rozen et. al., 1997); *Biastes brevicornis* (Panzer) (Rozen and Özbek, 2003). Information concerning ovary statistics is summarized in Rozen (2003). Descriptions of first instars of the Biastini have been lacking until now. Because we describe here the first instar of *Biastes emarginatus*, we append a comparative account of the first instar of *N. cressoni*. Mature biastine larvae have been described, as follows: *N. cressoni* (Rozen, 1966, 1996a; McGinley, 1981) and *R. rotundiceps* (Rozen et al., 1997). An intermediate stage larva of *Biastes emarginatus* was treated by Rozen (1993), as was the pupa of *N. cressoni* (Rozen, 1997).

BIOLOGICAL OBSERVATIONS

J.S. and K.R. made the following observations on *Biastes emarginatus* at a dense nesting aggregation of *Rophites quinquespinosus* Spinola in a large, unattended garden in Praha-Miškovice, a district of Prague, Czech Republic. This nesting site was discovered in 2006 and checked yearly since then. The *Rophites* nest aggregation occupied about a 3 $\times$ 3 m section of a 40–45° sloping surface on the sunny edge of a deciduous forest. More than 40 nests were observed there at a single time during the course of our observations. The site was partly shielded by the forest canopy from rain, particularly by one old linden tree (*Tilia cordata* Miller), but much of the area received considerable sunlight during a large part of sunny days. Ground cover, mostly tall grasses (ca 30 cm high), obscured much of the surface, so that many nest entrances were partly or completely hidden. Nest entrances, surrounded by tumuli when fresh, were left opened during foraging. The main burrows of host nests descended vertically with sharp turns to avoid rocks. Cells in a nest were arranged singly (i.e., not in linear series). Scattered nests were found a few meters away from the main site, but activity of *B. emarginatus* was low there. At least 10 *Biastes* females flew over the main part of the nest aggregation during the peak of activity on sunny days.

The aggregation was studied during 3–18 August 2008. *Rophites* females were active from early morning to late afternoon, flying even when the sky was overcast. In contrast, flight activity of *Biastes* depended on weather conditions. They flew only during warm, sunny, windless days (temperatures 25–30°C), and on such days they preferred host nests situated in sunny places. In addition, searching *Biastes* females moved with the sunny areas as these areas shifted during the day. Only during the warmest days, when the temperature rose over 30°C, did *Biastes* females prefer shaded parts of the nesting site. When the temperature fell below 25°C, the bees flew strictly in shafts of sunlight or were inactive, behaving similarly to the thermophilous behavior of another cleptoparasitic bee *Epeoloides coecutiens* (Fabricius) (Straka and Bogusch, 2007a). Because the site was completely shaded in the morning and late afternoon, *Biastes* females were active only from 10:30 A.M. to 4:00–5:00 P.M. (CEST).

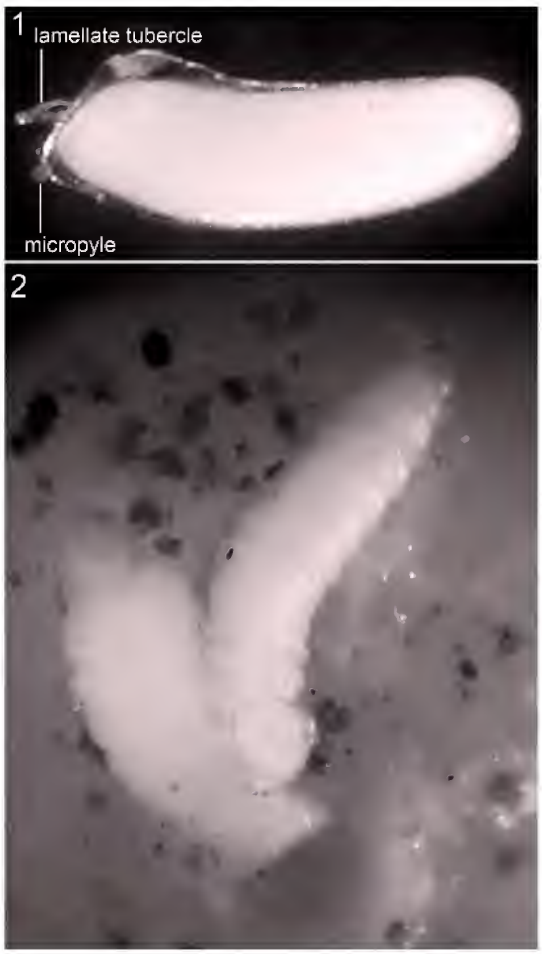
While searching *Biastes* females usually flew slowly close to the vegetation, scanning the ground surface for host nests. Because nests were often well hidden in the grass, *Biastes* would usually land when they spotted suspicious places and finish their inspections by crawling through the vegetation. The efficiency of finding nests seemed rather low, in that cleptoparasites usually spent a long time searching before finding active host nests. Females of *Biastes* always immediately enter a newly discovered nest and probably check its contents. Of 21 visits, most took only a very

short time (5–47 sec, on average 29 sec), probably indicating that the cell conditions were inadequate for successful egg laying. Seven attempts were probably successful as they took on average 1 min, 48 sec (the longest took 2 min, 30 sec). Interestingly, in about half these cases, the cleptoparasite waited 3–8 sec at the nest entrance before flying away, probably checking the conditions outside but risking confrontation with the host. If the entrance became shaded during the inspection, the parasite waited at the entrance several more seconds.

Parasitic behavior at the aggregation often resulted in conflict. Owners of active nests visit their nests many times a day, in that a female of *Rophites quinquespinosus* spends approximately 15 min inside her nest and 45 min foraging for every hour of daily activity. This means that the nest is protected at least one quarter of the time. Females of *Biastes emarginatus* fly away immediately when they encounter host females at the nests. A conflict situation happens when a host female returns while a *Biastes* female is inside the nest. The host grabs the parasite with her mandibles and throws her from the nest entrance.

No egg was found even when cell walls were carefully checked. The eggs could be well hidden or possibly laid on/in the spherical provision mass.

We encountered six first instars of *Biastes emarginatus* in four cells; two first instars were found on the surface of the food sphere in each of two cells and a single first instar was found in each of the other two cells. In one case of dual occupancy one larva appeared to be attacking the other (fig. 2). This discovery demonstrates that in two cases two eggs had been deposited in the chamber, but whether by a single female or separate females remains unknown. It almost certainly indicates that on hatching, this species, like most cleptoparasites, immediately rids the host cell of all but one individual, as is necessary if there is stored food adequate for only one individual.



Figs. 1, 2. Microphotographs of *Biastes emarginatus*. **1.** Mature oocyte, lateral view, showing transparent, glassy chorion. **2.** First instar apparently attacking another first instar.

OVARIAN STATISTICS AND MATURE OOCYTE

We dissected and later examined the ovaries of two adult females of *Biastes emarginatus* to obtain mature oocytes and data concerning the number of ovarioles and mature oocytes (summarized in table 1). The number of ovarioles is expressed by a notation that provides the number of ovarioles in each of a female's two ovaries. For example, the ovarian formula 4:4 refers to four ovarioles in each of the female's two ovaries; this number is the plesiomorphic number for the Apidae (Michener, 2007). One female of *B. emarginatus* possessed an ovarian formula of 7:6 (or 7) and the other female was 8:8. Although these counts are uncertain because of difficulty interpreting the maze of ovarioles on such small specimens, they confirm that

TABLE 1
Egg Indices, Number of Mature Oocytes, and Number Ovarioles of *Biastes emarginatus* Compared with Other Biastini
Figures in first three columns are averages; data for other Biastini from Rozen (2003: table 1).

Taxon	Egg index	Total mature oocytes Per specimen	Mature oocytes per ovariole	Ovariole formula	No. of specimens
Biastes emarginatus	0.30	14.5	1.00	7:6–7	2
				8:8	
Biastes brevicornis	0.18	32	1.60	~10:~10	1
Neopasites cressoni	0.31	14.5	1.16	6:6	2
				7:6	3
				5:6	1
Rhopalolemma rotundiceps	0.38	20.7	0.58[a]	17:14+[a]	3

[a]Based on one specimen.

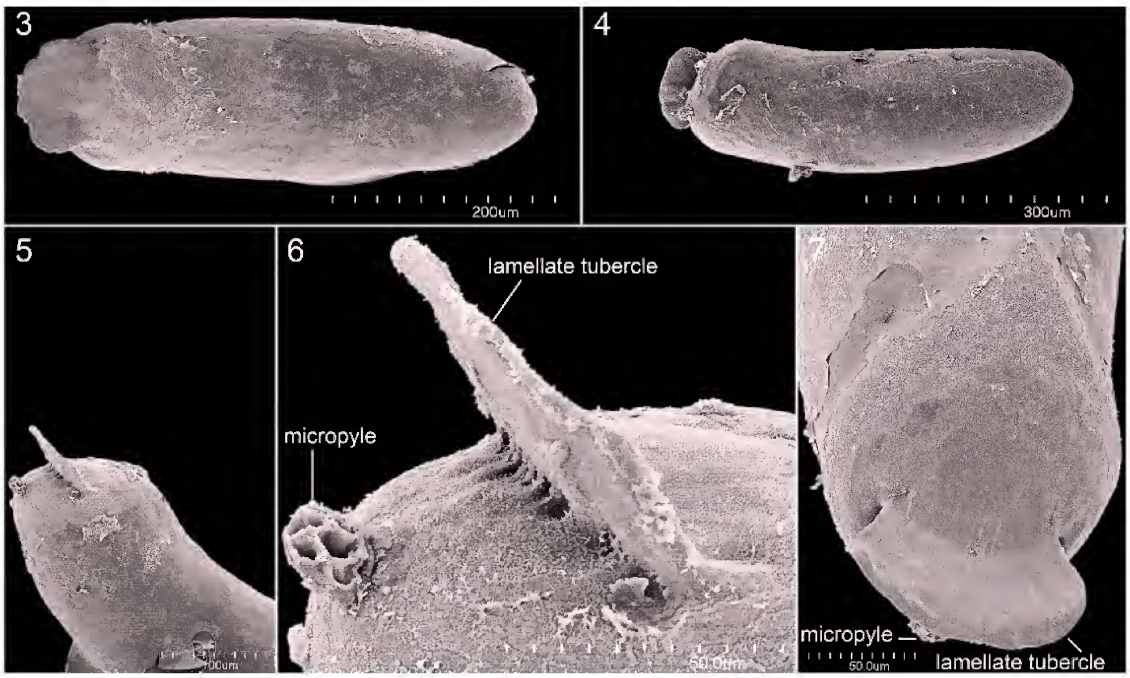
like most Nomadinae *B. emarginatus* has far more ovarioles per ovary than the plesiomorphic number (i.e., 4:4) found in many other Apidae (Rozen, 2003). The numbers for *B. emarginatus*, however, are lower than those found for *B. brevicornis* (see table 1 for comparisons with other known Biastini).

As also shown in table 1 the average number of mature oocytes in the two females of *Biastes emarginatus* appears to be 14.5, although it is difficult to determine with certainty which oocytes were completely encased by chorion. With this species, as with *B. brevicornis* (Rozen and Özbek, 2003) (and probably any species that has a thick chorion), there is a gradation in the development of the thickness of the chorion and especially of the anterior transverse lamellate tubercle. Although an oocyte is considered mature when it is surrounded by chorion, full deposition of a thick chorion comes later because it requires time after maturity and before oviposition.

The egg index of *Biastes emarginatus*, calculated by dividing the length of the largest oocyte (0.64 mm) by the distance between the outer rims of the female's tegulae (2.05 mm, 2.19 mm) (Iwata and Sakagami, 1966), is 0.29 or 0.31 (average 0.30) depends on which female is used. The index places the species well within the dwarf category (ibid.: table 2) with most of the other Nomadinae (Rozen, 2003: table 1).

The egg index of *Biastes emarginatus* is substantially higher than 0.18, the index of *B. brevicornis* (table 1). The meaning of this difference is not clear but might, in part, be an artifact of using egg length, a single dimension, as the criterion for volume, when in fact the mature oocyte of *B. brevicornis* is much wider than that of *B. emarginatus*. A further explanation may be forthcoming when eggs of the two species are found in situ in host cells. The eggs of *Neopasites cressoni* Crawford (Torchio et al., 1967) and *Rhopalolemma rotundiceps* (Rozen et al., 1997) are deposited so their dorsal surfaces are parallel to and flush with the host cell wall. Rozen and Özbek (2003) suggested the same arrangement for *B. brevicornis* because of the great thickness of the dorsal chorion of that species. However, the thick anterior chorion of the mature oocyte of *B. emarginatus* described below does not seems to fit that scenario. If the thick chorion functions to protect the egg of the cleptoparasite from the mandibles of a returning host female, chorion thickness only at the anterior end may suggest that the egg is "nailed," perhaps obliquely, into the cell wall or pollen mass with its anterior end partly exposed. A cleptoparasitic egg with only one end exposed in a host cell would more likely escape detection by a returning host female, than a large cleptoparasitic egg with its entire dorsal surface exposed: hence the need for *B. brevicornis* to have extremely small eggs, so as little chorion as possible is exposed to possible host detection. It will be interesting to learn more about this matter.

The following is formatted in accordance with the descriptions of the egg/mature oocytes of other cleptoparasitic bees in Rozen and Özbek (2003).



Figs. 3–7. SEM micrographs of mature oocytes of *Biastes emarginatus*. **3.** Entire oocyte, dorsal view, anterior end to the right. **4.** Same, approximate ventral view. **5.** Close-up of anterior end, near lateral view. **6.** Close-up of micropyle and lamellate tubercle from fig. 5. **7.** Anterior end of another oocyte, showing variation in shape of lamellate tubercle, anterodorsal view.

Mature Oocyte of *Biastes emarginatus*

Figures 1, 3–7

Diagnosis: The known eggs/mature oocytes of the Biastini show considerable chorionic variation when one compares *Rhopalolemma rotundiceps* (Rozen et al., 1997: figs 15, 19, 20), *Neopasites cressoni* (ibid.: figs. 18, 26), *Biastes emarginatus* (figs. 1, 3, 4), and *B. brevicornis* (Rozen and Özbek, 2003: figs. 17, 18). Despite these differences, as a group, they all uniquely possess a transverse median tubercle (described as lamellate in *Biastes*), which distinguishes them from all other cleptoparasitic bee eggs/mature oocytes studied to date (Rozen and Özbek, 2003; Rozen, 2003).

The much more elongate shape of the mature oocyte of *Biastes emarginatus* immediately distinguishes it from the stouter oocyte of *B. brevicornis*, as do the differences in thickened chorion in the two species (figs. 1, 3, 4; Rozen and Özbek, 2003: fig. 17). The broader, anteriorly projecting median lamellate tubercle arising from the semitruncate anterior end of the oocyte of *B. emarginatus* (figs. 5, 6) contrasts with the somewhat smaller, more dorsally projecting median lamellate tubercle on the rounded front end of the mature oocytes of *B. brevicornis* (Rozen and Özbek, 2003: figs. 17, 18).

Description: Length measured from posterior end to apex of lamellate tubercle/micropylar process 0.64 mm; maximum width lateral view, measured at right angle to long axis 0.18 mm; egg index 0.30. Shape (based on fully developed mature oocyte) elongate (approximately 3.5 × as long as maximum diameter), gently curved in lateral view (fig. 1), with presumed dorsal surface incurved; widest part in anterior half, rear part gradually, evenly tapering posteriorly until rounded posterior end; anterior end obliquely subtruncate, flattened, bearing micropylar process on lower edge and with large, thin but broad, lamellate tubercle extending from approximate center of truncation (as if to shelter micropylar process) (figs. 5, 6); anteri-

or edge of lamellate tubercles gently curved (fig. 7) to somewhat notched (fig. 3) in dorsal/ventral views; periphery of flattened anterior end without flange, such as found in many Ammobatini (Rozen and Özbek, 2003); micropylar process knoblike, elongate, forward projecting, with numerous holes (fig. 6). Under stereoscope, chorion clear, shiny, glassy, and faintly tinted where thick (fig. 1); chorion thickest only at front including flattened anterior surface and chorion just posterior to this surface; elsewhere chorion evident but evenly thin, without increased thickness of entire dorsal surface, thus unlike in *B. brevicornis* (Rozen and Özbek, 2003: fig. 17); under SEM examination smooth except with faint polygonal pattern.

Material Studied: Oocytes from two females, Czech Republic: Praha-Miškovice, 3-VIII-2008 (K. Rezkova and J. Straka lgt.).

Remarks: Although we had hoped to compare the mature oocytes of *Biastes emarginatus* and its host, *Rophites quinquespinosus*, the one preserved specimen of the host contained a single large oocyte, which apparently was still not mature.

FIRST INSTAR OF *BIASTES EMARGINATUS*

Figures 8–19

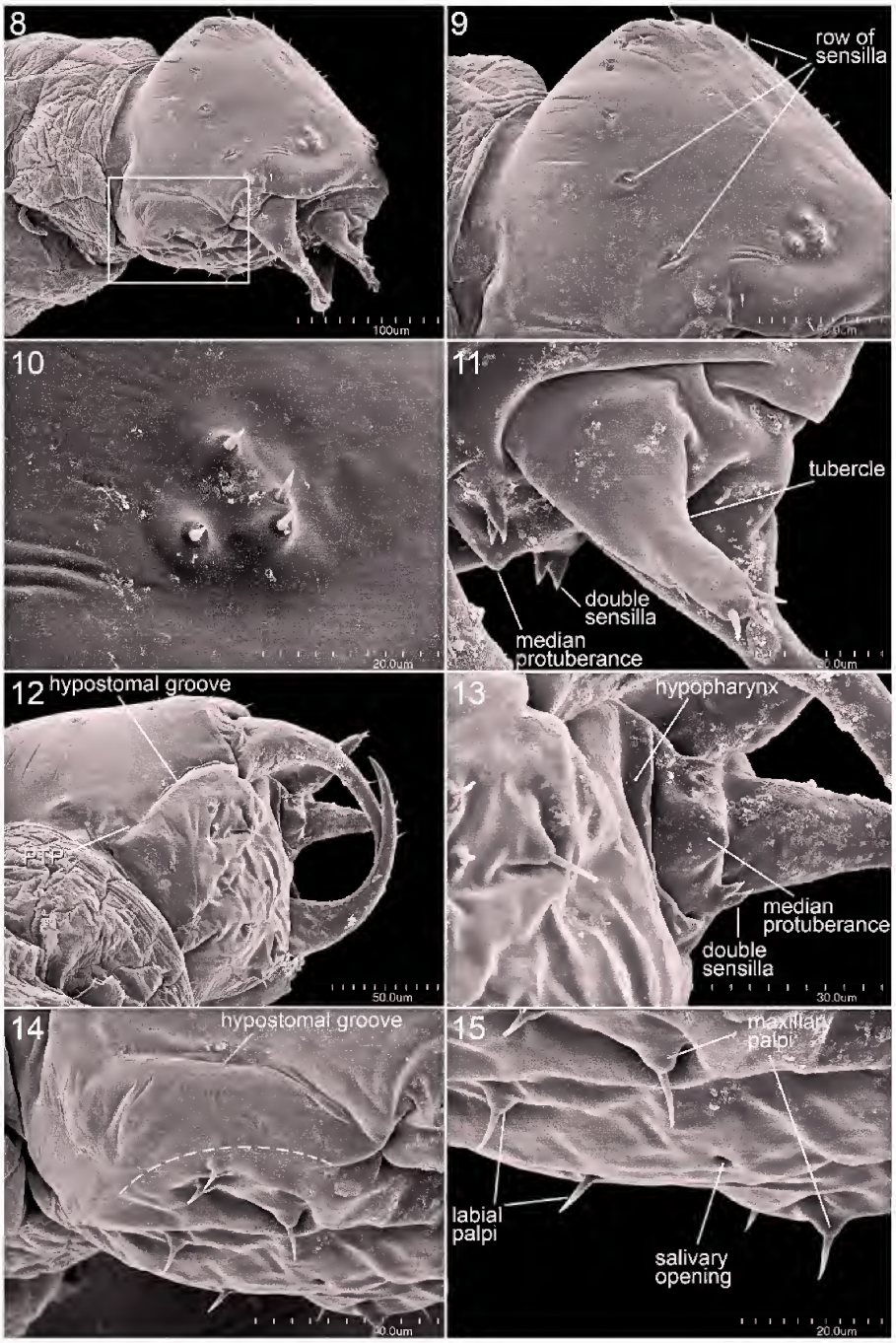
The format used here follows that in the descriptions of first instars of cleptoparasitic Apidae (Rozen, 1991). The only other available first instar is *Neopasites cressoni*, whose description is appended. These are the first SEM studies of any first-stage larva of the Nomadinae.

Diagnosis: Although slightly larger and with parietals somewhat more swollen compared with the first instar of *Neopasites cressoni* (description appended below), these two larvae are similar when examined by light microscope. The head shapes in lateral view appear to differ in that the front of the head of first-instar *Biastes emarginatus* in outline is straight as it rises to the curvature at the crown (fig. 18) whereas it is gently curved in *N. cressoni* (fig. 20). The paired labral tubercles of *B. emarginatus* are more slender than those of *N. cressoni*. Although the labiomaxillary region of *N. cressoni* may project ventrally more than that of *B. emarginatus*, this matter will need to be checked again when more material becomes available. Under limited SEM examination no feature could be found whereby the two taxa could be separated, thus leaving the impression of close agreement.

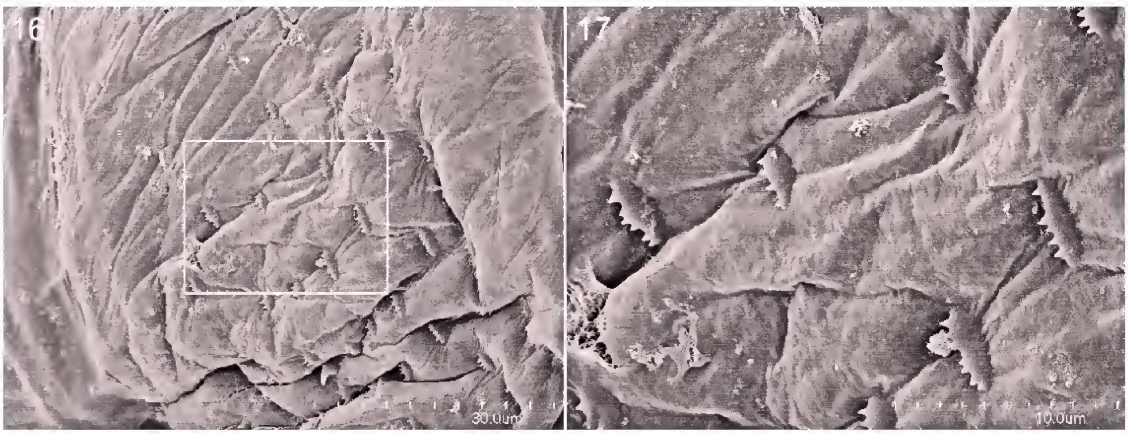
With the exception of its greatly reduced maxillary palpus, the first instar of *Biastes emarginatus* fits the description of the Nomadinae based on first instars in Rozen (1991).

Description: Length of newly eclosed larva 0.53 mm; other first stage specimens larger.

Head: Maximum transverse width of head capsule 0.19 mm; maximum transverse foramen width 0.11 mm. Shape slightly prognathous because of ventral surface extension of posteroventral constriction of parietals and presence of partial or complete postoccipital bridge (figs. 12, 14); head capsule not dorsoventrally flattened; parietals swollen laterally and dorsolaterally immediately in front of posterior constriction at rear of head capsule; vertex appearing bilobed because of median depression; in lateral view (figs. 8, 18) head shape similar to that of *Townsendiella pulchra* Crawford (Rozen, 1991: fig. 9). Integument of head capsule strongly sclerotized, moderately pigmented, as characteristic of hospicidal nomadine first instars; sclerotization ending at posterior margin of head capsule; capsule without ring of sharp-pointed spinulae across vertex as in Melectini (Rozen, 1991) but with linear row of simple setiform sensilla arising from shallow pits, extending across top of cranium from mandibular bases (fig. 9). Tentorium weak, not certainly complete; anterior tentorial pit not evident on SEM micrographs, but present as small spot above base of mandible somewhat mesad of imaginary line through antenna paralleling median line; posterior tentorial pits small put present (fig. 12); hypostomal groove/ridge present (figs. 8, 12, 14), darkly pigmented; sclerotization extending mesad (below in lateral view) of each hypostomal groove, presumably representing fused cardo and stipes, described below; pleurostomal and epistomal ridges not evident because of general thickness of integumental sclerotization; ecdysial line not



Figs. 8–15. SEM micrographs of head of first instar *Biastes emarginatus*. **8.** Entire head lateral view. **9.** Close-up of top of head showing position of antenna and linear row of sensilla across top. **10.** Close-up of antenna with 4 sensilla. **11.** Close-up of labrum, approximate lateral view. **12.** Ventral view of head from behind showing mandibles and labiomaxillary region. **13.** Close-up of labrum, approximate ventral view. **14.** Close-up of labiomaxillary region, identified by rectangle in fig. 8, dashed line demarking boundary of sclerotized (above) and membranous (below) areas. **15.** Close-up of membranous area of figure 14 identifying palpi and salivary opening. PTP = posterior tentorial pit.



Figs. 16, 17. SEM micrographs of first instar of *Biastes emarginatus*. **16.** Integument of first abdominal segment showing transverse, multidentate spicules. **17.** Close-up of area identified by rectangle in fig. 16.

evident; posterior margin of head capsule at approximate right angle to hypostomal groove in lateral view (figs. 8, 18). Antenna (figs. 8–10) small, scarcely protuberant, inconspicuous, with 3 or 4 sensilla. Labrum membranous except for pair of elongate, sclerotized tubercles apically beset with fine, bristlelike sensilla (figs. 11–13); epipharyngeal surface (figs. 11, 13) with small median protuberance beneath base of tubercles, laterad of which a pair of minute but elongate double sensilla on each side. Mandible (figs. 8, 12) elongate, many times longer than basal diameter, apically slender, tapering, curved, bearing a few sharply pointed sensilla at midlength but more at base. Labiomaxillary region scarcely protuberant in lateral view (figs. 8, 12, 18) with cardo and lateral part of stipes fused as single sclerite attached to parietal (figs. 8, 12, 14); posterior part of this sclerite extending mesad to form ventral postoccipital bridge or partial bridge to head (figs. 12, 14); rest of labiomaxillary region U-shaped, membranous, bearing maxillary and labial palpi as well as median salivary opening (figs. 14, 15); maxillary palpus (fig. 15) apparently a small tubercle with single long seta; two smaller setae (fig. 15) posterior to palpus presumably on maxilla, rather than on palpus; labial palpus a single seta on small tubercle posterior to and laterad of salivary opening. Hypopharynx apparently sclerotized with simple, curved anterior edge forming ventral edge of mouth (figs. 13, 19).

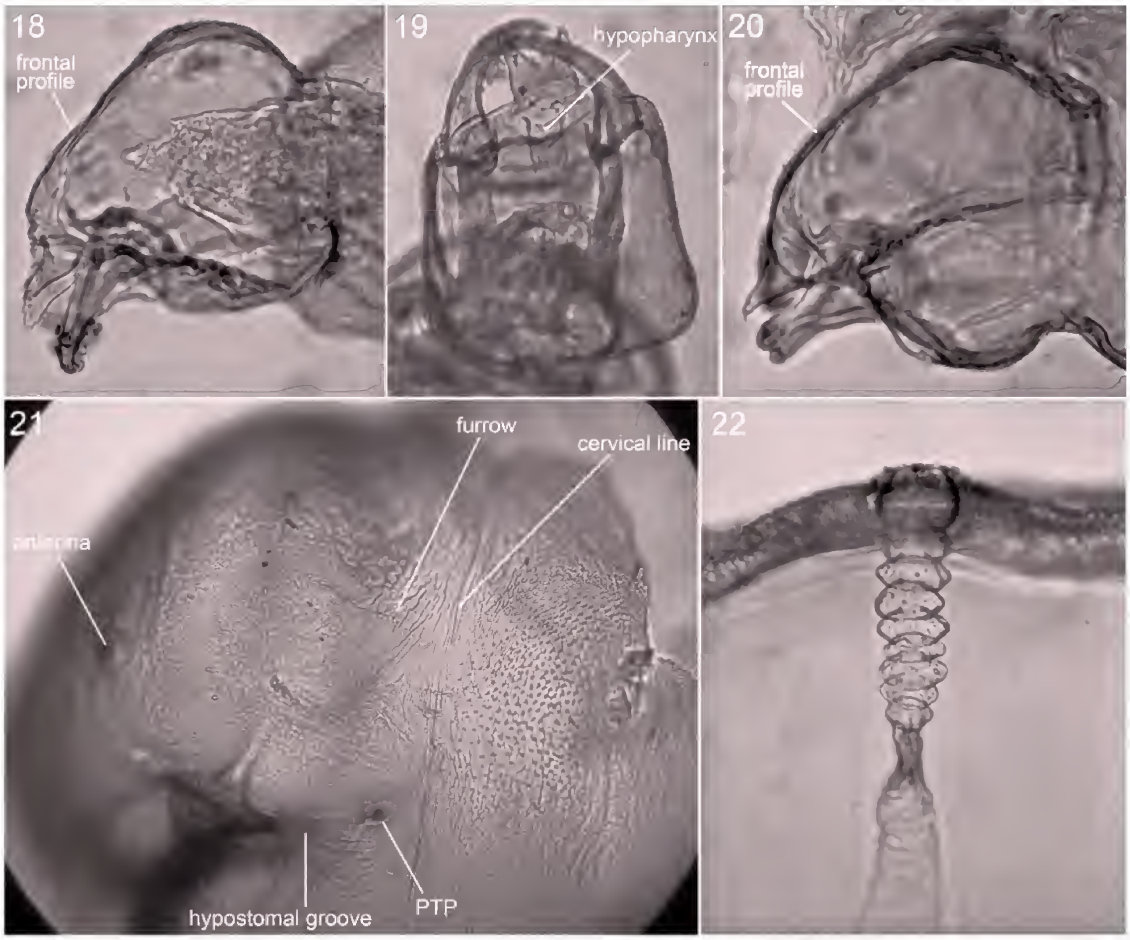
Body: Form elongate, tapering posteriorly (newly eclosed) to straight sided (after some feeding), without paired lateral tubercles on segments; abdominal segments 8–10 becoming progressively smaller, giving pointed appearance to posterior part of body; segment 10 small, bearing apically paired lobes, possibly eversible, presumably functional as pygopod. Integument with a few minute setae anteriorly but extensively covered, especially ventrally and laterally, with minute transverse, posteriorly sloping projections (thus cleatlike), each consisting of rows of 1–5 or 6 sharp points visible only with SEM (figs. 16, 17). Information concerning spiracles not available. Position of anus not identified.

Material Studied: Three first instars, Czech Republic: Praha-Miškovice, 3-VIII-2008 (K. Rezkova and J. Straka lgt.).

Remarks: The differences in body size among three specimens demonstrate the flexibility of the body wall to expand as well as a substantial intake of food during the first stadium.

The small, transverse, dentate, integumental projections (figs. 16, 17) presumably are modified spicules, which probably assist the larva in crawling as it searches for the host's offspring or larval competitors. Since similar projections occur on first instars of *Neopasites cressoni*, we wonder how extensively they will be found among other Nomadinae.

There is a striking resemblance among known first instars of *Neolarra* probably



Figs. 18, 19. Microphotographs of cleared head of *Biastes emarginatus*, lateral (18) and ventral (19) views, respectively. Fig. 20. Microphotographs of cleared head of *Neopasites cressoni*, lateral view, showing greater curvature to frontal profile. Fig. 21. Microphotograph of side of cleared head of mature larvae of *Biastes emarginatus*, showing texture of integument associate with rear part of head. Fig. 22. Microphotograph of spiracle of mature larva of *Biastes emarginatus*, side view. PTP = posterior tentorial pit.

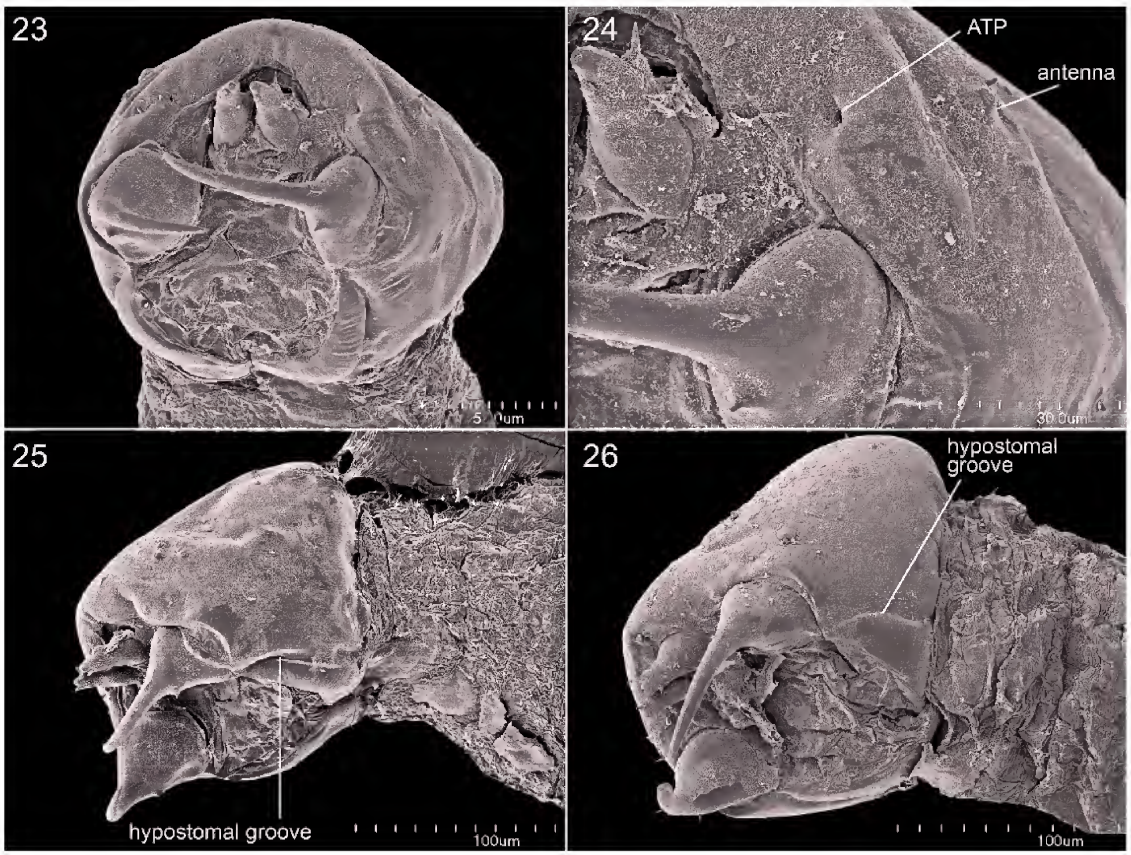
vigilans (Cockerell)[3], *Townsendiella pulchra* (Rozen, 1991), *Neopasites cressoni* (description appended), and *Biastes emarginatus* in the shape of the head in lateral view. It is created by the extreme elevation of the rear part of the head capsule resulting in a more-or-less straight lateral profile from the base of the clypeus to the highest point of the head capsule followed by an abrupt decline to the postoccipital ridge. Although it is tempting to speculate that these shared similarities are evidence of close relationships expressed in various cladograms in Rozen (1996a) and fig. 45 in Rozen et al. (1997), an examination of a broad spectrum of nomadine taxa suggests that a more thorough and detailed examination of their anatomy needs to be pursued before conclusions should be drawn.

[3] Specimen in the collection of the American Museum of Natural History.

POSTDEFECATING LARVA

Figures 21, 27–30

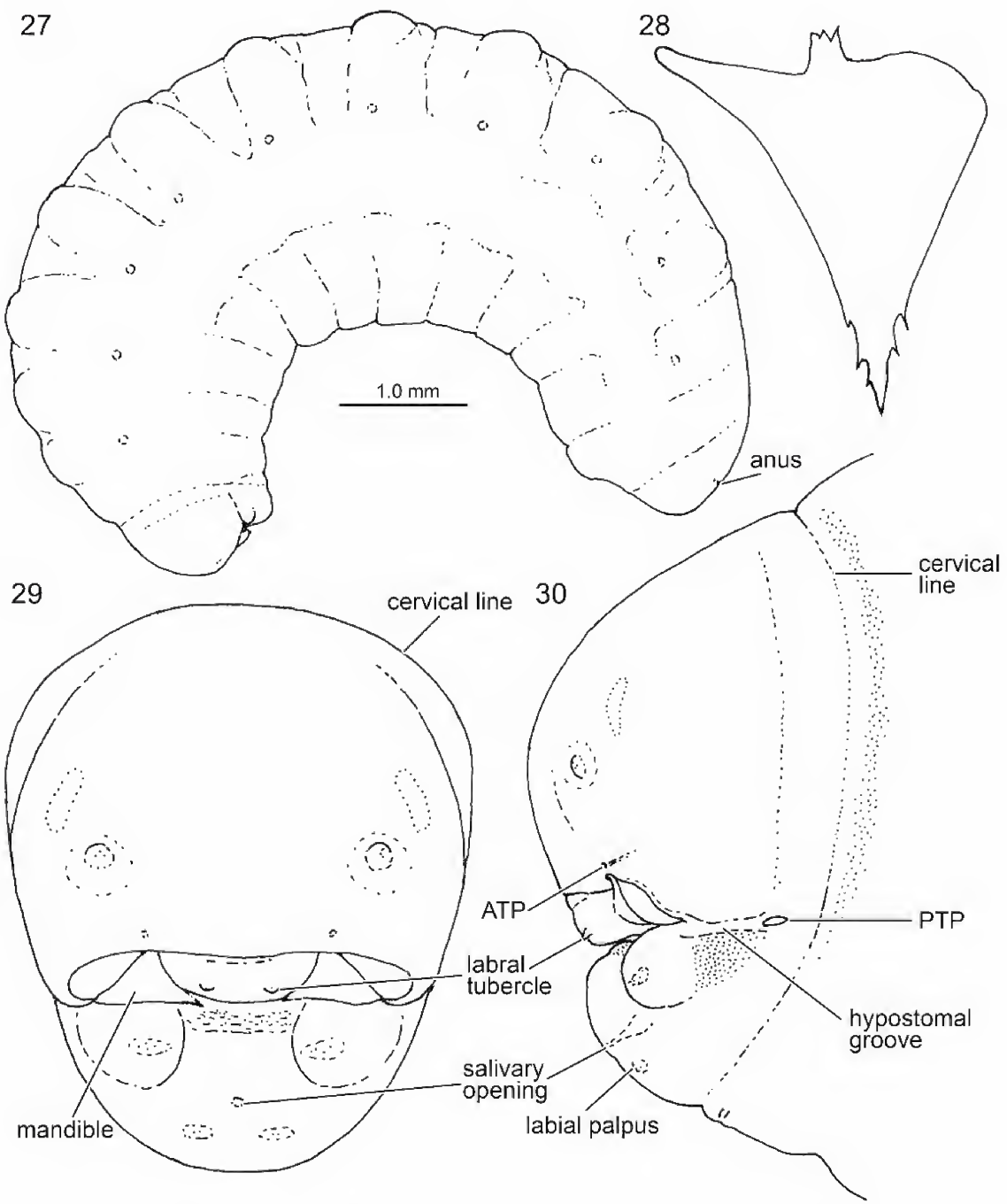
DIAGNOSIS: The mature larvae of the Biastini share a number of features with Neolarrini and Townsendiellini. Characters held in common by the three tribes include



Figs. 23–26. SEM micrographs of first instar of *Neopasites cressoni*. **23.** Head, near frontal view. **24.** Close-up of part of left side of head in fig. 23. **25.** Head, ventrolateral view. **26.** Head, of another specimen, approximate ventrolateral view. ATP = anterior tentorial pit.

the strongly produced frontal area on the head, recessed labroclypeal area in lateral view, apex of mandible thin and attenuate, maxillary palpus not projecting and nearly absent (visible as sensilla-bearing pale area on cleared head capsule), and protruding venter of abdominal segment 9 (abdominal segment 10 attached dorsally). In *Neolarra pruinosa* the anus is apical on a prolonged abdominal segment 10 (Rozen, 1966: fig. 79). The very short abdominal segment 10 (fig. 27) is another distinctive feature of the Biastini shared with *Townsendiella* (Rozen and McGinley, 1991: fig. 7), and, except for *Neopasites cressoni* (Rozen, 1966: fig. 72), the anus is positioned on the sloping dorsal surface of segment 10 (fig. 27). In *Townsendiella pulchra* the head capsule is extremely broad in frontal view with the top of the parietals higher than the vertex at midline (Rozen and McGinley, 1991: fig. 8), unlike the simple, evenly curved vertex of the Biastini (fig. 29). The mature larva of *Biastes emarginatus* will key successfully to *Rhopalolemma* in Rozen (2001). Whereas the paired dorsal body tubercles of *Neopasites cressoni* are scarcely evident (Rozen et al., 1997: fig. 32)[4], those of *B. emarginatus* and *R. rotundiceps* are low but obvious on most segments (fig. 27). The clypeus is strongly recessed in *R. rotundiceps*, so that labrum is produced slightly beyond the clypeus in lateral view (ibid., fig. 35) unlike in *B. emarginatus*.

[4] The tubercles seem more pronounced in the illustration of Rozen (1966: fig. 72), which questions the reliability of this character.



Figs. 27–30. Diagrams of postdefecating larva of *Biastes emarginatus*. **27.** Entire larva, lateral view. **28.** Right mandible, outer view. **29, 30.** Head, frontal and lateral views respectively. ATP = anterior tentorial pit; PTP = posterior tentorial pit.

DESCRIPTION: Length approximately 8.0 mm. **Head** (figs. 29, 30): Pigmentation, even of mandibles, weak; sensilla nonsetiform; much of integument minutely wrinkled; hypopharyngeal area, maxillae, and lateral parts of labium at base finely, extensively spiculate.

Head size moderately small compared with body; head capsule wider than long and dorsally rounded in frontal view; as seen in lateral view (fig. 30), frontal area projecting strongly forward, overhanging labroclypeal area. Tentorium nearly complete, extremely thin (dorsal arms probably missing); anterior tentorial pit extremely small, easily overlooked because of absence of epistomal ridge, situated close to but above and slightly mesad of anterior mandibular articulation, as in fig. 29; anterior tentorial arms extremely thin, tapering; posterior tentorial pit small, situated in hypostomal groove short distance (figs. 22, 30) in front of cervical line; posterior arm directed mesad, tapering, and apparently ending before reaching opposite one. Postoccipital ridge not evident, so that posterior margin of head capsule uncertain (as in *Rhopalolemma rotundiceps*; see description in Rozen et al., 1997) but possibly cervical line identified in figs. 21, 29, and 30, although this line passing well behind posterior tentorial pit and fading out toward bottom of head in lateral view; parietal with furrow[5] anterior to cervical line (also in *R. rotundiceps*) running roughly parallel to line and seemingly directed toward posterior tentorial pit; integumental texture (fig. 21) between furrow and cervical line finely, transversely wrinkled, nonspiculate, integument in front of area nonspiculate, with fewer wrinkles, none of which are transverse, area behind cervical line nonwrinkled and densely spiculate in most areas; area behind furrow flaring outward along sides of parietals, so that head in frontal view with two outlines laterally (fig. 29). Median longitudinal thickening of head capsule absent; hypostomal groove/ridge evident, short as measured from ventral mandibular articulation to posterior tentorial pit, fading out at pit, as seen in lateral view (figs. 21, 29); pleurostomal ridge weak, not clearly defined; epistomal ridge absent both between anterior tentorial pits and between pits and anterior mandibular articulations. Parietal band a faint integumental scar. Antennal prominence indistinguishable from forward projecting frontoclypeal region; antenna positioned normally on front of head capsule; antennal papilla, small but distinct, projecting, bearing 3–4 sensilla. Labrum short, small, recessed compared with projecting frontal region, without sclerite, with pair of very small, well separated tubercles on disc; epipharynx a short (because of short labrum), simple, curved surface.

Mandible (figs. 28, 29) extremely short so that when closed apex far removed from apex of apposing mandible, each ending beneath respective labral tubercle; mandible straight, apically tapering to fine point; dorsal and ventral apical edges with distinct, sharply pointed, apically directed fine teeth; in external profile, upper apical mandibular edge measured from basal articulation to apex much shorter than lower edged, as in *Neopasites cressoni* (Rozen, 1966: fig. 74); apex not curved toward mouth; cusp and apical concavity not differentiated. Maxillae and labium strongly fused into bulbous labiomaxillary region that in lateral view (fig. 30) is broadly attached to body. Maxillary apex slightly surpassed by labial/hypopharyngeal apex in lateral view (fig. 30); galea and maxillary sclerites including articulating arm of stipes absent; lateral integument of side of maxilla densely spiculate; maxillary palpus not projecting, represented by wrinkled integument bearing sensilla on cleared specimen. Labium not divided into pre- and postmentum; premental sclerite absent; labial palpus not projecting, below and laterad of salivary opening, similar to but smaller than maxillary palpus. Salivary opening simple, circular, without lips, situated below and posterior to labial/hypopharyngeal apex in lateral view (fig. 30); salivary duct pigmented, swollen shortly before opening. Hypopharyngeal groove faintly distinct (becoming unidentifiable on strongly sclerotized individuals); hypopharyngeal surface spiculate area dorsal to and behind nonspiculate labial apex.

Body (fig. 27): Without setae but with scattered nonsetiform sensilla especially on apices of paired dorsal tubercles; integument very finely spiculate in some area but spicules often difficult to identify because of pattern of

[5] This furrow is more linear in *Rhopalolemma rotundiceps* (Rozen et al., 1997: fig. 35).

fine, transverse wrinkles that tend to be parallel except on apices of dorsolateral tubercles, where wrinkles are multidirectional; integument without spines or sclerotized tubercles. Body form moderate in robustness, not quite as slender as that of *Neopasites cressoni* or *Rhopalolemma rotundiceps* (Rozen et al., 1997: figs. 32, 33); intersegmental lines weakly incised between most body segments; dorsolateral intrasegmental lines not evident; each thoracic segment with weakly evident, paired dorsolateral tubercles; abdominal segments 1–7 with paired dorsolateral tubercles somewhat more evident but absent on segments 8–10; all dorsolateral tubercles moderately low, conical (as opposed to transverse); venter of abdominal segment 9 in lateral view (fig. 27) appearing somewhat produced because of dorsal attachment of segment 10; segment 10, short with length much less than one-half height in lateral view (fig. 27), attached dorsally to 9 (fig. 27); anus a transverse slit on sloping dorsal surface of 10 (fig. 27); perianal area with small dorsal lip immediately above anus that is to width of anus. Spiracles (fig. 22) subequal and moderate in size, projecting beyond body wall, with rims; peritreme present, moderately wide; atrium globular, pigmented; atrial wall without denticles or rings; primary spiracular opening with collar; subatrium moderately short, in side view tapering from body surface inward, with about 8 chambers. Male with median integumental scar toward posterior margin of abdominal segment 9; female sex characters unknown.

Material Studied: Six postdefecating larvae, Czech Republic: Praha-Miškovice, 1-IX-2007, 18-VIII-2008 (K. Rezkova and J. Straka lgt.); host: *Rophites quinquespinosus*.

Remarks: The description of an immature larva of this species from Switzerland (Rozen, 1993), though far less complete, is in agreement with the above description.

DISCUSSION AND CONCLUSIONS

Similarity in morphology both of first instars and of mature larvae among the tribes Biastini, Neolarrini, and Townsendiellini may indicate a close relationship of these bees. This similarity was recognized several times in cladistic analyses (Alexander, 1990; Rozen, 1996a; Rozen et al., 1997) with high bootstrap support for the Neolarrini-Biastini relationship (Townsendiellini not included) (Straka and Bogusch, 1997b). Five characters listed in the diagnosis of mature larvae can be expanded by a specific combination of adult characters shared by bees of these three tribes: propodeal profile with a nearly horizontal basal zone, a gonocoxite with an internal septum, and the presence of a ventral parapennial lobe with strong setae of the gonocoxite. The only bee with a similar adult character combination is *Hexepeolus rhodogyne* (Linsley and Michener); however, its mature larvae are largely different (see Rozen, 1996b).

Biastini, *Townsendiella*, and *Neolarra* probably form a well recognizable clade like the morphologically diverse clades within the tribes Ammobatini, Ammobatoidini, Brachynomadini, and Epeolini. However, a comprehensive phylogenetic analysis based on bee morphology and possibly DNA sequences is needed.

The nest-searching behavior we describe here for *Biastes emarginatus* is similar to that of many small nomadine cleptoparasites that attack nests of ground-nesting solitary bees. Like many cleptoparasitic bees that deposit their eggs in cells that are still open and thus likely revisited by host females, their eggs/mature oocytes are small and possess a thick, presumably protective chorion against attack by host females. The configuration of the chorion suggests that the eggs may be embedded in the pollen sphere and/or the cell wall, to make them less obvious to host females. The first instars are small as probably dictated by the small egg size and, like those of other Nomadinae, possess adaptations for seeking out and battling eggs/larvae of the host or of competing cleptoparasites. These adaptations consist of a heavily sclerotized head capsule, large, curved, sharply pointed mandibles, elongate labral tubercles, and the ability to crawl, presumably with the aid of a terminal pygopod and transverse, cleatlike spicules (reported here for the first time) on the body surface.

As with many cleptoparasitic bees with specialized hospicidal early instars, the larval instars of *Biastes emarginatus* exhibit a sub-

stantial anatomical modification between the hospicidal stage and subsequent instars, which presumably are devoted to consuming provisions. One would assume that the anatomy of the last larval instar would reveal obvious, perhaps plesiomorphic features for ingesting provisions, such as scoop-shaped mandibles powered by mandibular muscles attached to the parietals, and parietal architecture that supports associated muscular contraction for biting into the provisions. As an example we clearly see this is the situation with *Coelioxys* (Megachilidae) (e.g., Baker, 1971). However, this does not seem to be the case for *Biastes emarginatus*, *Neopasites cressoni*, and likely for some (if not many) other Nomadinae. With *B. emarginatus*, the mandibles of the last instar are so short that in repose their apices are far separated, they are not scoop shaped, and the head capsule is thinly sclerotized and so week that not only is it not constricted behind (suggesting weak musculature), but the posterior margin of the capsule cannot be certainly identified (is it the cervical line or the anterior, faint furrow that is directed toward the posterior tentorial pits? if the former, how then do we account for the anterior furrow?). The postoccipital ridge is absent. The intriguing questions are: how does this animal feed and how do we explain its peculiar morphology? The answers will probably come from fieldwork: observing them as they feed, not only the first and last instars, but the intermediate ones as well. Because mandibles of the last instar seem ineffectual for chewing into food and forcing the food into the esophagus, might an internal study of this structure reveal some sort of cibarial or esophageal pump, thereby explaining the rotund projection of the front of the head capsule?

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APPENDIX

First Instar of *Neopasites cressoni*
Figures 20, 23–26

The first instar of *Neopasites cressoni* is described here to allow a comparison with the first instar of *Biastes emarginatus*. The description is based mostly on a cleared specimen, but the SEM micrographs are of two of seven specimens found dead in a single cell, presumably having been killed earlier by other cleptoparasites. While heavily sclerotized areas were relatively unaffected, membranous areas, including the labrum and labiomaxillary regions shrank and became distorted through postmortem processes. Many setae were also broken, for reasons not understood.

Diagnosis: See Diagnosis of first instar of *Biastes emarginatus* above.

Description: Length of newly eclosed specimen 0.44 mm.

Head: Maximum transverse width of head capsule 0.18 mm; maximum transverse foramen width 0.13 mm. Shape about as described for *Biastes emarginatus* except foramen magnum not nearly as constricted and parietals not swollen as greatly dorsolaterally, so the median depression of vertex less exaggerated, only faintly lower than lateral parts of vertex in frontal view; integument of head capsule about as described for *Biastes emarginatus*; capsule clearly with simple setae, not spinulae but arrangement uncertain. Tentorium weak, not certainly complete; anterior (fig. 24) and posterior tentorial pits present; hypostomal groove present, darkly pigmented; sclerotization mesad (below) of each hypostomal groove present as in *B. emarginatus*; other features of parietal about as described for *B. emarginatus*. Antenna (fig. 24) as described for *B. emarginatus*. Labrum as described for *B. emarginatus* except paired tubercles stouter at base and information about epipharyngeal surface unknown. Mandible as described for *B. emarginatus*. Labiomaxillary region probably much like that of *B. emarginatus* because of fusion of cardo with lateral parts of stipes while rest of region remaining membranous except perhaps membranous part more ventrally protuberant although SEM micrographs (figs. 23, 25, 26) show this area badly desiccated due to postmortem deterioration; posterior part of fused cardo/stipes sclerite forming ventral postoccipital bridge (fig. 23), except bridge perhaps not fused medially; identification of maxillary and labial palpi as well as median salivary opening uncertain (figs. 23, 25, 26). Hypopharynx apparently sclerotized as in *B. emarginatus*.

Body: Form elongate, linear, as described for *Biastes emarginatus*. Integument with a few minute setae anteriorly and with minute, transverse, posteriorly sloping projections consisting of rows of 1–5+ sharp points visible only with SEM, as in *Biastes emarginatus* (fig. 17). Spiracular peritreme flush with body wall (fig. 25). Position of anus unknown.

Material Studied: Seven first instars, USA: Arizona: Cochise Co., 3 mi SW of Rodeo, Hidalgo Co., New Mexico, V-1-1965 (J.G. Rozen), presumably all collected dead; one first instar, same data except IV-27-1966 (J.G. Rozen, M. Favreau), collected alive.

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